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Citation for published version:

Sargison, N, Frances, E, Davison, C, Bronsvort, M, Handel, I & Mazeri, S 2016, 'Observations on the biology, epidemiology and economic relevance of rumen flukes (Paramphistomidae) in cattle kept in a temperate environment', *Veterinary Parasitology*, vol. 219, pp. 7-16.
<https://doi.org/10.1016/j.vetpar.2016.01.010>

Digital Object Identifier (DOI):

[10.1016/j.vetpar.2016.01.010](https://doi.org/10.1016/j.vetpar.2016.01.010)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Veterinary Parasitology

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Observations on the biology, epidemiology and economic relevance of rumen flukes (Paramphistomidae) in cattle kept in a temperate environment.

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Graphical Abstract



Highlights

- Estimates of the proportion of animals affected by rumen fluke
- Description of the distribution of rumen fluke in the stomach
- Estimates of the performance of rumen fluke egg counting at different times of year
- Investigation of the relationship between growth rates and rumen fluke infection

ABSTRACT

There is concern about the probable recent introduction, increased prevalence and potential economic impact of rumen fluke infection of United Kingdom cattle. A study of 339 cattle slaughtered in a Scottish red meat abattoir was undertaken with the aims of describing the prevalence and geographical distribution of rumen fluke infection, estimating its effect on production, and evaluating faecal egg counts (FECs) as a tool to diagnose infection in live animals and study the epidemiology of the disease. The overall proportion of cattle consigned to the abattoir from northern United Kingdom with rumen fluke infection in the forestomachs was 0.29. Rumen flukes were distributed predominantly in the cranial sac of the rumen and adjacent to the reticular

groove. Overall, a mean of 213 and median of 44 rumen flukes was identified in the forestomachs of rumen fluke-positive cattle. The mean and median FECs of animals were 26.01 and 5.20 eggs per gram (epg), respectively. There was a significant difference between the mean FECs per rumen fluke of 0.08 and 0.13 epg during summer/autumn and winter sampling periods, respectively. The overall correlation between rumen fluke FECs and the number of flukes in the forestomach was high, albeit lower in the summer/autumn than in the winter period. The sensitivities of rumen fluke FECs for the identification of flukes in the forestomach during the summer/autumn and winter sampling periods were 0.65 and 0.85, respectively. These results will aid in the interpretation of rumen fluke FECs when monitoring cattle health and production and studying the parasite's epidemiology in a temperate environment, thereby informing rational, precise and sustainable disease control.

Keywords: rumen fluke; epidemiology; *Calicophoron*; abattoir; FEC; cattle; Impact

1. Introduction

Rumen flukes are digenean trematodes belonging to the family Paramphistomidae, which has several common genera of livestock parasites including *Calicophoron* and *Paramphistomum*. The parasites have heteroxenous life cycles involving water or mud snail intermediate hosts. Ciliated miracidia that hatch from fluke eggs shed in the faeces of final hosts penetrate their snail intermediate hosts and then multiply asexually to develop into cercariae. Cercariae emerging from the intermediate hosts encyst as metacercariae that are the infective stage for the final hosts. In the final hosts, the immature larval stages first establish in the proximal small intestine where they feed on the mucosa, before migrating proximally through the abomasum to the forestomach, and developing to egg laying adult parasites (De Waal, 2010).

Rumen fluke parasitism is considered to be important in many parts of the world, in particular in subtropical regions (Taylor et al., 2007), in livestock and ruminant wildlife kept in environments that support the intermediate mud or water snail hosts, but until about 2007 was uncommon in temperate regions, and considered to be very rare in the United Kingdom and Republic of Ireland.

However, rumen flukes, now known to be *Calicophoron daubneyi* (Gordon et al., 2013) have since become commonplace in United Kingdom (Foster et al., 2008) and Irish (Murphy et al., 2008; Zintl et al., 2014; Toolan et al., 2015) cattle, while their prevalence has increased in mainland European cattle (Mage et al., 2002), renewing interest in their biology, epidemiology and economic importance.

There is a paucity of information about the biology of parasitic stages of *C. daubneyi* in intermediate and final hosts and of free living egg, miracidial, cercarial and metacercarial stages in the environment, under temperate environmental conditions. Diagnostic tools are required to enable understanding of each of these aspects at a farm-specific level under different specific environmental, climatic and host management conditions, and to predict the risk of disease thereby informing sustainable control strategies. For example, knowledge of the relationship between adult parasite burdens and faecal egg shedding under specific environmental and management conditions may help to predict the role of animals as reservoirs of infection, or the level of cercarial challenge to the intermediate hosts, thereby informing the need for precise, strategic anthelmintic treatments.

An understanding of the ecology of the intermediate hosts and interactions with other trematode parasites is required as a basis for appropriate, sustainable parasite control strategies. While the intermediate host for *C. daubneyi* in many European regions is accepted as being *Galba truncatula* (Rieu et al., 2007), the snail intermediate hosts for rumen flukes in different temperate environments in the United Kingdom have not been conclusively established. *G. truncatula* is the principal intermediate host for the liver fluke, *Fasciola hepatica*, which is a common and important cause of production loss in United Kingdom and Irish ruminant livestock (Sargison and Scott, 2011; Gordon et al., 2012). In France, the introduction and apparent spread of paramphistomosis has been putatively attributed to the efficacy of fasciolosis control leaving the intermediate host, *G. truncatula*, free to become infected with *C. daubneyi* (Rieu et al., 2007). Nevertheless, this scenario seems unlikely in the United Kingdom, as control of fasciolosis is hampered by undiagnosed or inappropriately managed triclabendazole resistance (Sargison and Scott, 2011) and compounded by effects of climatic change (Kenyon et al., 2009; Fox et al., 2011).

Diarrhoea, weight loss and deaths have been attributed to heavy infection with immature stages of rumen flukes in the intestine (Millar et al., 2012) and migrating through the mucosa of the abomasum of British cattle. Oxyclozanide is the only drug licensed in the United Kingdom and Republic of Ireland that is effective against rumen flukes and there is a common perception amongst producers of improved health of infected yearling cattle following such treatment. However, this perception is generally not supported by production data and to-date, while occasional deaths have been reported (Millar et al., 2012), a widespread causal relationship between rumen fluke infection and clinical disease has not been conclusively established. Nevertheless, farmers have understandably focused attention on grazing management and anthelmintic drug treatments for rumen fluke control, to insure against unknown or hidden production costs, such as reduced milk yields in cows or impaired food conversion in growing animals, associated with the increased prevalence of rumen flukes in their herds. The consequential widespread use of oxyclozanide may prove to be irrational, due to a paucity of knowledge concerning the biology and epidemiology of rumen flukes under specific environmental, climatic and management conditions. Furthermore, while oxyclozanide is also licensed for the treatment of fasciolosis, it is only effective against adult stages of *F. hepatica*, hence the common dependence on the drug as a general flukicidal anthelmintic may compromise the control of fasciolosis.

Adult rumen flukes are generally considered to be important in cattle kept in hot and wet regions (Rolfe et al., 1991; Rangel-Ruiz et al., 2003), but mostly harmless in European cattle (Rieu et al., 2007). Production loss has been associated with heavy burdens of adult *Paramphistomum* sp. in experimentally infected sheep (Rolfe et al., 1994) and *C. microbothrium* in experimentally infected cattle (Mavenyengwa et al., 2005). However, in natural infections, effects of rumen flukes may be confounded by concurrent infections with other helminth parasites, in particular *F. hepatica* (Dorny et al., 2011), which has similar intermediate host, environmental and climatic requirements.

The study of rumen flukes in live animals depends on the use of faecal egg counts (FECs). However, these detect only patent infections and their interpretation is constrained by the paucity of information about how they relate to parasite

burdens and pathology. Information collected at abattoirs can be used for cross-sectional studies to compare animals' management and performance with enumeration of parasites and other indices of infection. This can enable the estimation of diagnostic test performance, for example FECs, as a prerequisite for their use in the field to understand the parasites' epidemiology. Abattoir-based studies have been used as a component of the study of the phylogenetics of *C. daubneyi* in Irish cattle (Zintl et al., 2014) and to describe various aspects of rumen fluke infection in Spanish (Arias et al., 2011; González-Warleta et al., 2013; Ferreras et al., 2014), Belgian (Malrait et al., 2015) and French (Rieu et al., 2007) adult cows. However, rumen fluke infection of final hosts is considered to be cumulative, hence observations of the prevalence and biology of the parasites in these studies may represent both recent infection and burdens established over previous years (González-Warleta et al., 2013). Furthermore, confounding effects of fasciolosis in these studies may differ from those in the United Kingdom, where *F. hepatica* is a particular established major constraint.

In this paper we describe a study using information pertaining to prime cattle slaughtered in a large Scottish red meat abattoir during summer/autumn and winter periods, with the aims of: i) describing the distribution of rumen flukes in the forestomachs; ii) investigating the relationship between rumen fluke egg counts and parasite burdens; iii) estimating the prevalence of rumen fluke in cattle slaughtered at the abattoir (abattoir prevalence); iv) describing the geographical distribution of rumen fluke in cattle slaughtered at the abattoir and the correlation with co-infection with *F. hepatica*; and v) investigating the potential economic impacts of rumen flukes, hence the rationale for treatment.

2. Materials and Methods

2.1. Study resources

Data were collected from a total of 339 finished prime cattle slaughtered in a large red meat abattoir in central Scotland. All cattle slaughtered in United Kingdom abattoirs are uniquely identified and traceable under the auspices of the British Cattle Movement Services (BCMS) (British Cattle Movement Services), enabling collation of management information. Information about breed, age and origin prior to slaughter was collected to accompany parasitological data for

168 cattle slaughtered during the winter (on 5 sampling days between 13th January and 3rd March 2014), and 171 cattle slaughtered during the summer/autumn (on 5 sampling days between 25th August and 6th October 2014). Data were collected for every tenth animal slaughtered on each sampling day, representing the entire day's throughput. This strategy was designed both for logistical reasons, and to reduce the risk of disproportionate collection of multiple animal samples from the same holdings (representing farms), thereby allowing a representative distribution of cattle origins of consignment and management systems.

2.2. Parasitological sampling

Each animal's unique identification was checked and recorded at the point of slaughter. Carcasses and corresponding forestomachs and livers were tagged to allow samples to be collected from the correct animals and matched to BCMS data. Livers were inspected by the Food Standards Agency's Meat Hygiene Service (Food Standards Agency) for evidence of fasciolosis, and examined in detail by us as part of a separate study. Forestomachs were incised along the greater curvature of the rumen and everted to remove their contents, as a standard part of the abattoir's tripe preparation process. Everted tagged forestomachs were arranged in a predetermined manner in dorso-medial and ventro-lateral planes as shown in Fig. 1 for enumeration and mapping of the distribution of rumen flukes. Between 1 and 100 rumen flukes from each parasitised animal, depending on the numbers of flukes present, were fixed in 70% ethanol and archived for future studies.

Faecal samples corresponding to tagged carcasses were collected immediately following the evisceration process, ensuring accurate identification of the collected material. Fluke egg counts were performed using a quantitative sedimentation method. The samples were mixed using a spatula and 5 g weighed out in a measuring cylinder. Water was added up to the 40 ml mark and contents were mixed using a stirring rod. Contents were sieved through a tea strainer and collected in a 250 ml beaker for removal of coarse faecal material. The contents were then sieved through a 150 μ m sieve, collected into a narrow bottomed glass tube and allowed to sediment for 3 min. Excess liquid was

removed and sediment was transferred into a 15ml conical bottomed tube and allowed to re-sediment for 3 minutes. Excess liquid was syphoned off and the sediment was transferred onto a petri dish. One drop of 0.5 % methylene blue was added to stain the background material and eggs were identified using a stereoscopic dissecting microscope, counted and divided by 5 to enumerate as eggs per gram (epg). Rumen fluke eggs were differentiated from *F. hepatica* eggs on the basis of their shape and the absence of bile staining of the former.

2.3. Data analyses

R (Version 3.0.3) (R Core Team, 2014) was used for most of the statistical analysis in this study. Rumen fluke infection abattoir prevalence estimates with exact binomial confidence intervals were provided for each sampling season and overall. An animal was classed as positive if one, or more, rumen fluke was identified in the rumen. Spearman's correlation coefficients were estimated for the correlation between the number of flukes identified in the forestomach and the number of epg in the faeces using package *RVAideMemoire* (Hervé, 2015). Estimates of the sensitivity and specificity (and exact binomial confidence intervals) of FEC were obtained by comparing binary results of FEC with the results of rumen fluke counts in the forestomach. An epg >0 was considered as a FEC positive result. Estimates were also provided for the overall arithmetic mean epg per fluke count, and for each sampling period. A Mann-Whitney U test was used to compare the mean results of each sampling period. In order to investigate the association between rumen fluke and *F. hepatica* infection an Odds Ratio and 95% CI was estimated using package *Epicalc* (Chongsuvivatwong, 2012).

The association between rumen fluke infection and carcase characteristics was investigated in the following way. A Mann-Whitney-Wilcoxon test was used to investigate the difference in carcass grade and fatness score between animals infected with rumen fluke and rumen fluke-free animals. Generalised linear regression modeling, using package *lme4* (Bates 2014), was used to estimate the association between rumen fluke infection and carcase weight/age at slaughter as a proxy for growth rate, with and without adjusting for other important

factors including sampling period, breed and concurrent liver fluke infection as fixed effects, and holding (farm of origin of consignment) as a random effect.

Package `ggplot2` (Wickham, 2009) was used for drawing plots. Packages `maptools` (Bivand and Lewin-Koh, 2014), `maps` (Becker and Wilks, 2013), `mapdata` (Becker and Wilks, 2014) and `GISTools` (Brunsdon and Chen, 2014) were used to draw maps of the geographical distribution of both rumen and liver fluke infections among the sampled animals. The spatial scan statistic of the Bernoulli model (Kulldorff, 1997) was used to identify spatial clusters with increased relative risk of being rumen fluke positive, using SaTScan software version 9.2 (Kulldorff, 2009).

The distribution of rumen flukes in the forestomachs was described by allocating numbers of flukes identified to defined anatomical regions and calculating their density based on the estimated area of the forestomach wall in each region. Two dimensional sketches of necropsy specimens shown in Fig. 1 were digitised and the proportional area of each anatomical region was estimated using QGIS 2.8.1 mapping software (QGIS Development Team, 2015). More precisely, polygons were drawn based on the sketches and the area was calculated using vector geometry tools. The total area estimated was added up and each layer was assigned a relative area based on its estimated area over the total estimated area.

3. Results

3.1. Animals

Samples were examined or collected from 339 cattle consigned from 154 separate holdings (168 cattle during the winter period and 171 during the summer/autumn period from 78 and 92 holdings, respectively). During both sampling periods, the animals were consigned from holdings in cattle-finishing regions throughout Scotland, Northern Ireland and northern England and comprised of five different standard breeds or their crosses plus other types as shown in Table 1. The age of sampled animals ranged from 369 to 1081 days old with a mean of 705.9 days old during the winter period and from 383 to 1095 days old with a mean of 748.5 days old during the summer/autumn period. Their carcase weights ranged from 185.6 to 494.9 kg with a mean of 339.3 kg and from 242.4 to 471 kg with a mean of 345.2 kg during winter and

summer/autumn sampling periods, respectively, thereby representing a range of different animal production systems. Histograms of age and carcase weight by rumen fluke status are shown in Fig 2.

3.2. Parasitology

3.2.1 Abattoir prevalence of rumen flukes

Rumen flukes were found in the forestomachs of 97 out of 343 cattle (abattoir prevalence 0.29, 95% CI 0.24 – 0.34). There was no significant difference in the prevalence of rumen flukes, as determined by positive identification of parasites, in the forestomachs of cattle slaughtered during the summer/autumn and winter sampling periods, being 0.29 (95% CI 0.22 – 0.36) and 0.29 (95% CI 0.22 – 0.36), respectively.

3.2.2 Number and distribution of rumen flukes in the forestomachs

Among animals classed as rumen fluke positive the number of parasites identified in each forestomach ranged from 1 to 2530, with a mean value of 213.2 and a median of 44. During the winter sampling period the number ranged from 1 to 2530 with a mean of 294.1 and a median of 61.5. During summer/autumn the number ranged from 1 to 1470 with a mean of 133.9 and a median of 25. This difference was statistically significant (Mann-Whitney U test p-value of 0.004).

Fig. 3 shows scatterplots and confidence intervals of rumen fluke density per forestomach compartment during each sampling period. Overall, the highest densities of rumen flukes were seen in the cranial sac of the rumen and adjacent to the reticular groove. The average numbers of rumen flukes per unit area (related to the total area of the 2 dimensional diagram of the forestomach) were: caudal rumen pillars, 0.36; cranial sac of the rumen, 6.97; dorsal sac of the rumen, 0.30; reticular groove, 3.93; reticulum, 0.85; and ventral sac of the rumen, 1.02.

3.2.3 Correlations between FECs and rumen fluke burdens

The number of rumen fluke epg identified in faeces of FEC positive animals ranged from 0.2 to 220.4 with a mean of 26.01 and a median of 5.20. During the

winter sampling period this ranged from 0.2 to 220.4 with a mean of 35.64 and a median of 10.70. During the summer/autumn sampling period this ranged from 0.2 to 171.6 with a mean of 14.24 and a median of 2.00. This difference was statistically significant (Mann-Whitney U test p-value of 0.005).

The overall mean FEC per rumen fluke was 0.11 epg. The mean and median epg per fluke during the summer/autumn were 0.085 and 0.019, respectively. The mean and median epg per fluke during the winter were 0.126 and 0.108, respectively. Comparison between the two periods using the Mann Whitney U test gave a p-value of less than 0.001. The Spearman's correlation coefficients for the relationship between the number of rumen fluke epg identified using FEC and the number of rumen flukes identified in the forestomach during the summer/autumn and winter sampling periods were 0.78 (95% CI 0.66 - 0.86) and 0.9 (95% CI 0.83 - 0.95), respectively. This can also be seen graphically in Fig. 4.

3.2.4 Diagnostic value of rumen fluke FECs

Table 2 shows a cross tabulation of binary rumen fluke FEC results vs. identification of the parasites in the rumen. The estimate of the sensitivity of FECs for the diagnosis of rumen fluke infection in cattle, based on the examination of the forestomach as a gold standard, was higher in the winter than in the summer/autumn (Table 3).

3.3 Epidemiological findings

Rumen flukes were identified in cattle consigned from most regions from which finished animals were procured. The identification of rumen fluke infected cattle was over-represented in animals consigned to the abattoir from high rainfall regions such as Northern Ireland, central Scotland, south-west Scotland/north-west England and north-east England. Rumen flukes were identified in cattle from the same regions in central and south-west Scotland, north-east and north-west England and Northern Ireland from which *F. hepatica* was identified. According to Fig. 5, cattle that were infected with either rumen flukes or *F. hepatica* were overrepresented in certain localities. This is further supported by the higher-risk clusters identified by SatScan using the spatial scan statistic (Fig.

5). Among cattle infected with rumen flukes, 45% were co-infected with *F. hepatica* (Table 4). In fact, the odds of cattle being infected with rumen fluke were estimated to be 3 times higher (Odds Ratio 3.11, 95% CI 1.81 - 5.31) in cattle infected with *F. hepatica* compared with liver fluke free cattle (chi-squared p-value <0.001).

3.4 Potential economic implications of rumen fluke infection

Results of a univariable regression analysis modeling the association of rumen fluke and other important factors with growth rate are shown in Table 1. Univariable analysis shows that rumen fluke has a statistically significant (p-value = 0.006) negative association with carcass weight/age at slaughter as a proxy for average lifetime growth rate. However, when adjusting for important factors including sampling period, breed, liver fluke status and holding (farm) as random effects, this effect is not statistically significant (p-value = 0.38), albeit still negative (estimate = -0.011). Addition of rumen fluke as a variable in a model containing all the other factors did not improve the Akaike information criterion (AIC), supporting the conclusion that there was no statistically significant association between the presence of rumen flukes in the forestomachs and growth rate (Table 5).

Most of the rumen fluke positive cattle in our study had relatively low forestomach fluke counts (Fig. 6) when compared to reliable reports from hot and wet regions (Rolfe et al., 1991; Mavenyengwa et al., 2005).. Consequently, there were insufficient animals with high enough rumen fluke burdens to enable statistically validated assessment of effects of the size of the rumen fluke burdens on growth rates. There was no statistically significant association between rumen fluke infection and carcass grades (using the EUROP system, where E represents excellent and P represents poor conformation), or fatness scores (on a scale of 1 to 5, where 5 is very fat), with Mann-Whitney-Wilcoxon p-values of 0.061 and 0.657, respectively.

4. Discussion

In light of our identification of rumen fluke infection in about 29% of the finished prime cattle slaughtered in the large Scottish red meat abattoir between January

and October 2014, the concerns of farmers in the United Kingdom about the importance of the parasites in their cattle are understandable. The overall abattoir prevalence of rumen fluke infection in this sample of 0.29 (95% CI, 0.24 – 0.34) was higher than the levels that had been previously reported based on the abattoir identification of flukes in the forestomachs of Spanish and Portuguese cattle of 0.12 (Arias et al., 2011), 0.19 (González-Warleta et al., 2013) and 0.06 (Ferrerías et al., 2014), but similar to the level reported in a small-scale French abattoir survey of 0.39 (Rieu et al., 2007). However, each of these previous abattoir surveys included adult cows, which would have had a greater opportunity to acquire higher parasite burdens than the mostly younger cattle in the current study (Ferrerías et al., 2014). The abattoir prevalence of cattle infected with rumen fluke in our study is similar to that reported in Ireland, based on the percentage of diagnostic laboratory faecal submissions that were positive for paramphistome eggs between 2004 and 2013 (Zintl et al., 2014) and a combined veterinary surveillance and abattoir study between 2013 and 2014 (Toolan et al., 2015). However, direct comparisons between these studies are not valid because very different methods were employed. Nevertheless, similarities and differences in prevalence of rumen fluke infection probably reflect the extent and availability to grazing cattle of environments that are suited to the free living stages of the parasites and intermediate mollusc hosts. The environments from which cattle were consigned to the Spanish abattoirs were described as being cold to temperate and dry (Ferrerías et al., 2014), whereas the regions from which most of the infected animals were consigned to the Scottish abattoir had temperate and wet climates. The median number of parasites identified in the forestomachs (44) of the finished prime cattle slaughtered in our study was lower than the numbers reported in adult Spanish cattle, in which between 100 and 400 (Arias et al., 2011), 266 (González-Warleta et al., 2013) and 144 (Ferrerías et al., 2014) rumen flukes were reported. In contrast, the burdens of *C. calicophorum* and *Paramphistomum ichikawai* in a subtropical location in eastern Australia were measured in thousands, with FECs up to 4,000 epg (Rolfe et al., 1991). We did not undertake conclusive molecular species-specific parasite identification because all of the rumen flukes identified in European cattle since 2007 have been shown elsewhere to be *C. daubneyi*

(Gordon et al., 2013; González-Warleta et al., 2013; Zintl et al., 2014; Malrait et al., 2015; Toolan et al., 2015).

Our study enabled accurate description of the distribution and densities of rumen flukes in the forestomachs of cattle. Observations of the highest density of rumen flukes in the cranial sac of the rumen and adjacent to the reticular groove, were in agreement with previous descriptions of the distribution of rumen flukes in Spanish cattle (González-Warleta et al., 2013; Ferreras et al., 2014). The high density of flukes in the ruminal atrium and close to the reticular groove might suggest that most do not move far from the site of their origin in the forestomach, having completed their migration from the proximal small intestine, or may simply reflect a predilection for attachment to the base of the ruminal papillae (Fuertes et al., 2015) in those areas. The low density of flukes in the dorsal sac of the rumen and caudal ruminal pillars might reflect the absence of papillae in these areas, and, or, the natural apposition of these areas to the ruminal gas cap, creating a zone from which the parasites cannot feed. The clinical significance of these observations is uncertain.

Better understanding is needed of the relationship between adult rumen fluke burdens and faecal egg shedding by cattle kept under specific management conditions in a temperate and wet environment, in order to predict the level of cercarial challenge to the intermediate hosts, thereby informing rational control strategies. Our Scottish abattoir study provided information about the diagnostic performance of FECs to enable reliable estimation of the adult rumen fluke burdens of live cattle. Rumen fluke FECs were reasonably well correlated to the burdens of flukes in the forestomach, but the overall mean FEC of about 0.1 epg per rumen fluke was notably lower than the FECs of about 1 epg per adult rumen fluke extrapolated from the results of previous French (Rieu et al., 2007) and Belgian (Malrait et al., 2015) abattoir studies. However, the methods used in these studies for the enumeration of fluke eggs and determination of forestomach parasite burdens differed from our study. Modified McMaster and mini-Flotac egg counting methods were used in the French and Belgian studies, respectively. In the French study, the forestomach fluke burden was recorded on a three point ranking scale, from which a count was estimated, while in the Belgian study the forestomachs were washed prior to numerical estimation of

the burden. The French and Belgian studies were of adult cows, in which the rumen fluke burdens would have been cumulative, while our study involved only younger prime cattle, most of which would only have experienced one summer/autumn grazing period. Furthermore, any unknown confounding effects of fasciolosis on egg output of rumen flukes would have differed between the study populations. Unlike the French (Rieu et al., 2007) and Belgian (Malrait et al., 2015) studies, in which a reduction in faecal egg output per adult fluke was reported with higher burdens of more than 100 and 200 flukes, respectively, we were unable to show such differences because too few cattle having high rumen fluke burdens were identified to allow for statistical validation.

Faecal egg counting is the only practical test that is validated for the identification of rumen fluke infection in live animals, but it can only diagnose patent infections. Knowledge of the sensitivity and specificity of FECs to identify trematodes in live animals is necessary to validate their application in the monitoring and diagnosis of disease and hence enable planned parasite control. In this study we have used identification of rumen flukes in the forestomachs as a gold standard, acknowledging that this approach will miss early infections where immature parasites have not reached the forestomachs. The overall sensitivity of 0.75 (95% CI, 0.65-0.83) and specificity of 0.97 (95% CI, 0.94-0.99) of FECs to identify the presence of rumen flukes in the forestomachs of cattle slaughtered in the Scottish abattoir was lower than the sensitivity of 0.94 (95% CI, 0.81-0.99) and specificity of 0.98 (95% CI, 0.92-0.99) reported following a Belgian abattoir survey of 125 cows, sampled on two days during late November and December (Malrait et al., 2015). The inferred difference in sensitivities may reflect differences in the parasite burdens of the cows sampled in the Belgian study and younger cattle slaughtered at the Scottish abattoir, as well as the different sampling periods and differences in the FEC and fluke counting methods used. The validity of our results may be further supported by the strong correlations observed between FEC and parasite counts in the forestomach.

The correlation between rumen fluke FECs and the burdens of flukes in the forestomach was significantly stronger during the winter sampling period than during summer/autumn. Furthermore, the lower sensitivity of FECs for the

diagnosis of rumen fluke infection of the forestomach of cattle during the summer/autumn than during the winter, in conjunction with the significant difference in mean FEC per fluke between the sampling periods, and our identification of a higher density of smaller parasites (a subjective observation that was not included in the analyses) close to the reticular groove during the former sampling period, would be consistent with the presence of immature parasites in the forestomach. Importantly from a disease control perspective, these observations might indicate that the peak timing of migration of immature stages through the abomasum and reticular groove to the forestomach broadly coincided with the summer/autumn sampling period.

The over-representation of rumen fluke infected cattle consigned to the abattoir from regions with known high rainfall supports the role of environmental factors in determining suitable environments for the transmission of infection between definitive and intermediate hosts, and for the mollusc intermediate hosts themselves. It is possible that some cases of rumen fluke infection that were identified in cattle consigned from low rainfall, cereal cropping regions where many beef finishing systems are based may have spent a previous grazing season in the wetter western regions, but unfortunately this could not be investigated using the available data. The high level of co-infection with rumen flukes and *F. hepatica* liver flukes, and overlapping geographical distribution of farms from which higher densities of cattle infected with either parasite were consigned to the abattoir, would suggest that the environmental conditions favouring the intermediate hosts for rumen flukes are similar to those required by the known *G. truncatula* intermediate hosts for *F. hepatica*. While the species identity of mollusc intermediate hosts for rumen flukes in the United Kingdom has not been definitively demonstrated, these observations would be consistent with reports of the most important intermediate host for *C. daubneyi* in mainland Europe being *G. truncatula* (Augot et al., 1996; Rieu et al., 2007) and the report of identification of *C. daubneyi* stages in *G. truncatula* in Wales (Jones et al 2015). Co-infection with *C. daubneyi* and *F. hepatica* has also been shown in French cows, albeit at a lower frequency (Rieu et al., 2007) than we identified in the cattle consigned to the Scottish abattoir. The fact that rumen flukes were identified in some cattle that were not infected with *F. hepatica* and *vice versa*

might simply reflect previous animal movements or effective treatments, but also raises intriguing questions about the conditions, ecological niches and parasite interactions that might give rise to *G. truncatula* snails becoming infected with one parasite or the other. Our results, therefore, show the need for further study of the biology of rumen flukes in their snail intermediate hosts to improve understanding of the epidemiology of rumen flukes in cold to temperate environments as a basis for appropriate, sustainable parasite control strategies.

An understanding of the impact of rumen fluke infection upon cattle production is needed in order to prioritise trematode species-specific anthelmintic drug treatments. Direct study of disease caused by immature rumen fluke stages is prevented by the lack of a routine diagnostic test for the presence of immature flukes in live animals. However, regression analysis of relationship between average lifetime growth rates and forestomach fluke burdens helps to show the economic impact of both adult and immature flukes. There is a widely held, but unproven assumption that high burdens of several hundred or more adult flukes in the forestomach influence faecal consistency and cause production loss (Rolfe et al., 1991; Mavenyengwa et al., 2005; Dorny et al., 2011; Malrait et al., 2015) as a consequence of damage to the ruminal papillae caused by parasite attachment by their ventral suckers (Fuertes et al., 2015). However, in our study, multivariable regression analysis showed no statistically significant effect of forestomach rumen fluke infection status on growth rates when adjusting for other important factors such as *F. hepatica* co-infections. Additionally, based on subjective visual assessment (results not shown) no relationship between rumen fluke burden and damage to the ruminal papillae was observed. In fact, denudation of the papillae was seen in many animals both with and in the absence of rumen fluke infection, putatively associated with ruminal acidosis in grain-finished cattle.

While we did not demonstrate decreased production in rumen fluke infected cattle, we did show the confounding effect of concurrent infection with *F. hepatica* and need for caution when interpreting growth rate trends in relation to a single parasite. In a previous abattoir study in Spain (Arias et al., 2011), 20% of liver fluke affected animals were also infected with rumen fluke. In our study, we observed even higher levels of co-infection with almost half of the

animals infected with liver flukes, being also infected with rumen flukes. This suggests that liver and rumen fluke infections might have similar epidemiologies in temperate regions. Therefore teasing apart the effect of each infection on growth rates is complex. The use of multivariable regression modeling, to estimate the effect of rumen fluke infection, while accounting for liver fluke infection among other important animal related factors, allowed us to overcome this problem. In the absence of demonstration of production loss attributable to rumen fluke, British and Irish farmers' dependence on the use of oxclozanide as a general flukicide is irrational, whenever treatments are given at times of year when immature stages of *F. hepatica*, against which the drug is ineffective, are present. Young cattle that are thriving poorly, or diarrhoeic might nevertheless benefit from oxclozanide treatment if they have larval paramphistomosis.

We have shown the manner in which data collected at an abattoir can be used to improve understanding of: i) the prevalence of rumen fluke infection of cattle; ii) the distribution of rumen flukes within the forestomach of cattle; iii) the sensitivity and specificity of rumen fluke FECs in determining the presence of parasites in the forestomach; iv) the geographical distribution of rumen fluke infection in northern United Kingdom cattle consigned to a large Scottish abattoir; and v) the economic impact of rumen fluke infection. Our approach could be scaled up to enable risks of infection to be determined associated with different geographical, climatic and environmental conditions, which may affect parasite epidemiology (González-Warleta et al., 2013). This could potentially provide a basis for predictive models of disease to aid in the implementation of sustainable parasite control measures. Having shown the relationship between rumen fluke burdens in the forestomachs and faecal rumen fluke egg shedding, research into the effects of specific environmental conditions and management practices is now needed to inform the parasite's epidemiology. Further planned research is also indicated to validate the economic impact of rumen fluke infection in cattle kept in cold to temperate environments, where co-infection with *F. hepatica* is commonplace.

Acknowledgements

We acknowledge Scotbeef Limited who fully funded this project and allowed us to carry out the sampling and collect material. We acknowledge our colleagues who helped with sampling as well as the slaughterhouse workers and the Meat Hygiene Service for making this work possible.

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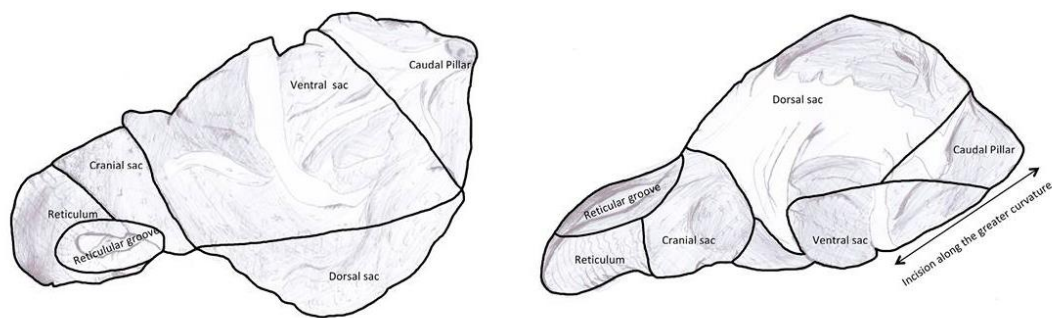


Fig. 1. Schematic representations of everted tagged forestomachs. Forestomachs were viewed in dorso-medial (left) and ventro-lateral (right) planes and the schematics were used for enumeration and mapping of the distribution of rumen flukes.

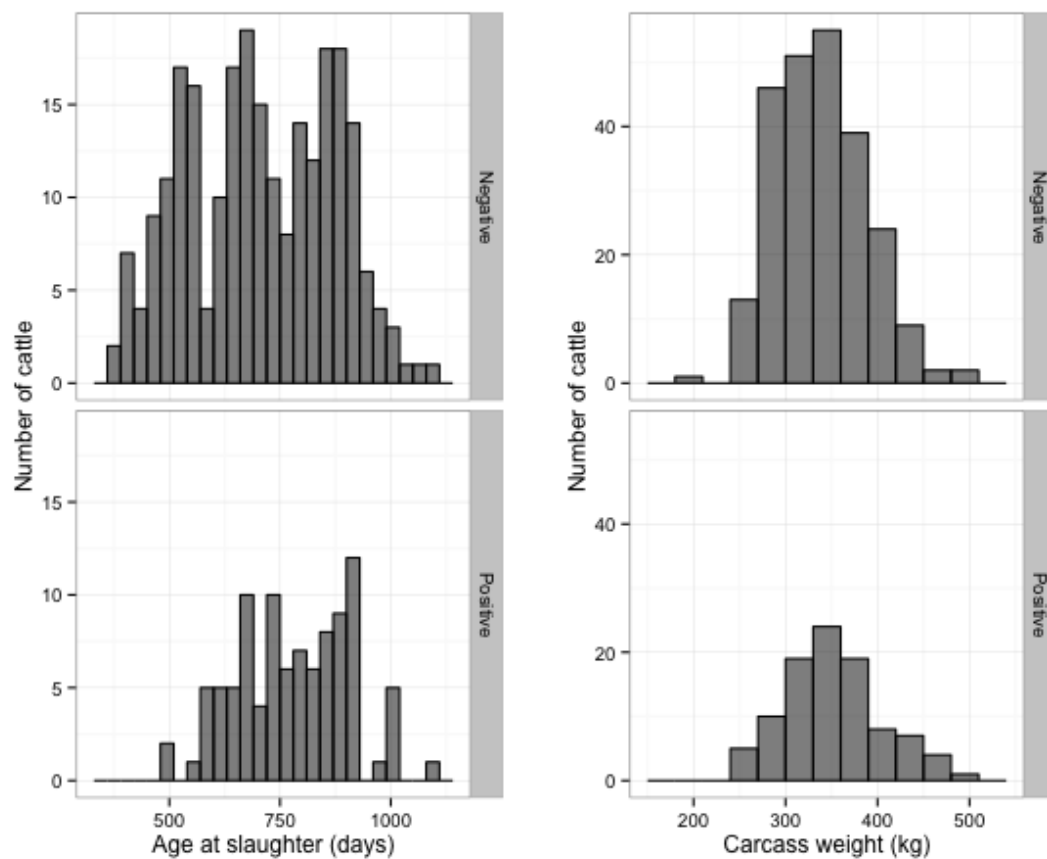


Fig. 2. Age at slaughter (left) and carcass weights (right) by rumen fluke status of cattle slaughtered and sampled during the summer/autumn and winter periods.

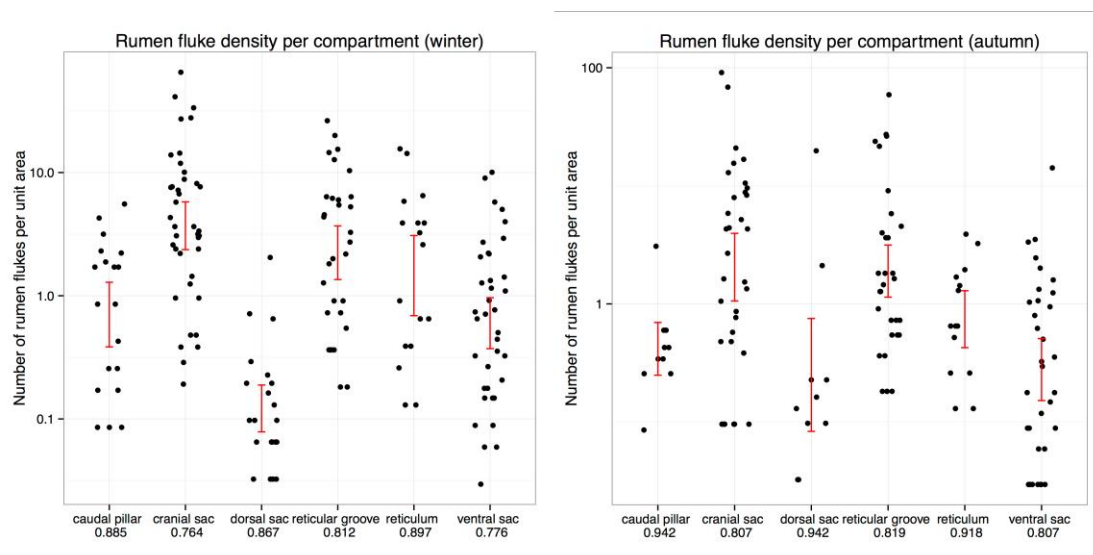


Fig. 3. Scatterplots of rumen fluke density per forestomach compartment. These are shown during the winter (left) and summer/autumn (right) sampling periods with confidence intervals shown in red. Values below each compartment name indicate the percentage of forestomachs with no parasites in that specific compartment.

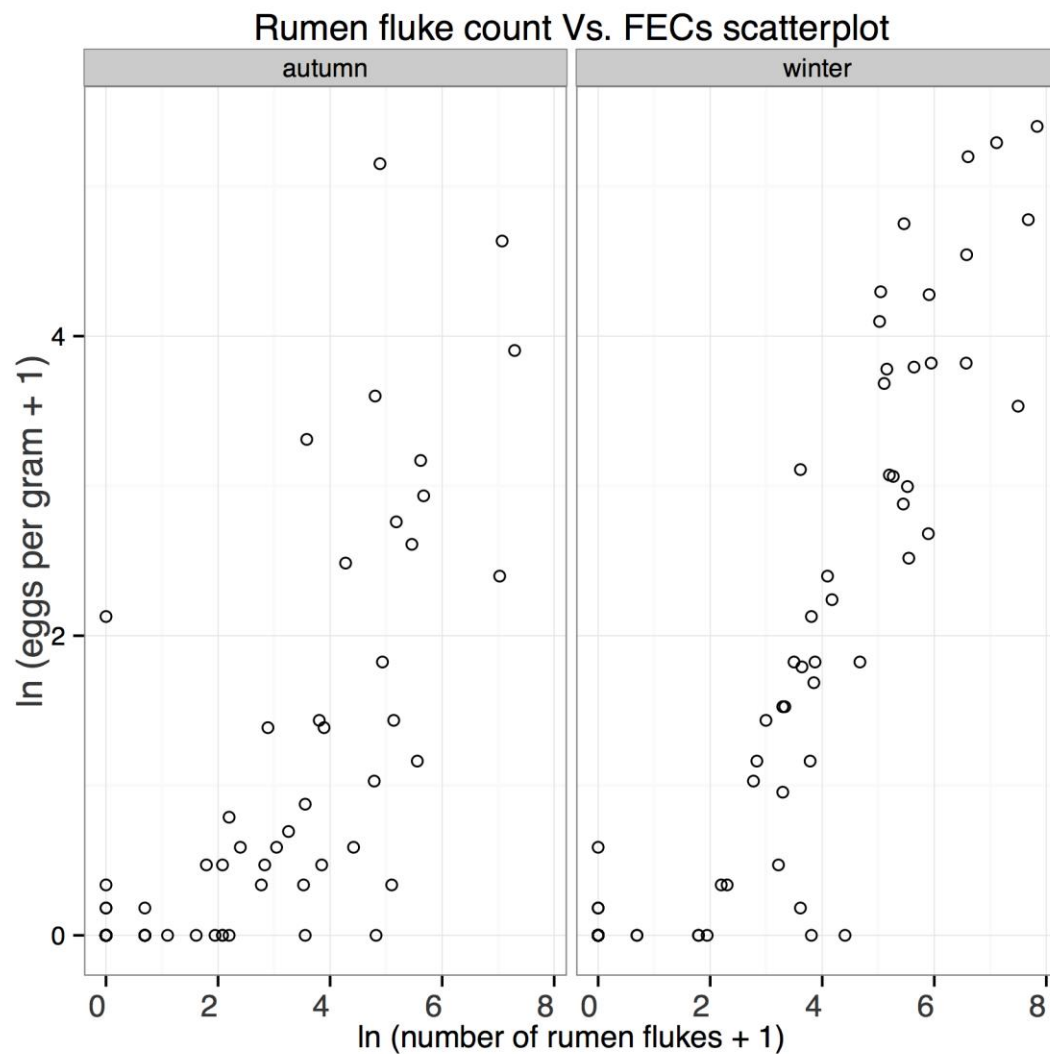


Fig. 4. Scatterplots of FEC epg Vs rumen fluke count. These are shown during autumn (left) and winter (right). The natural logarithms of the original variables plus 1 are shown.

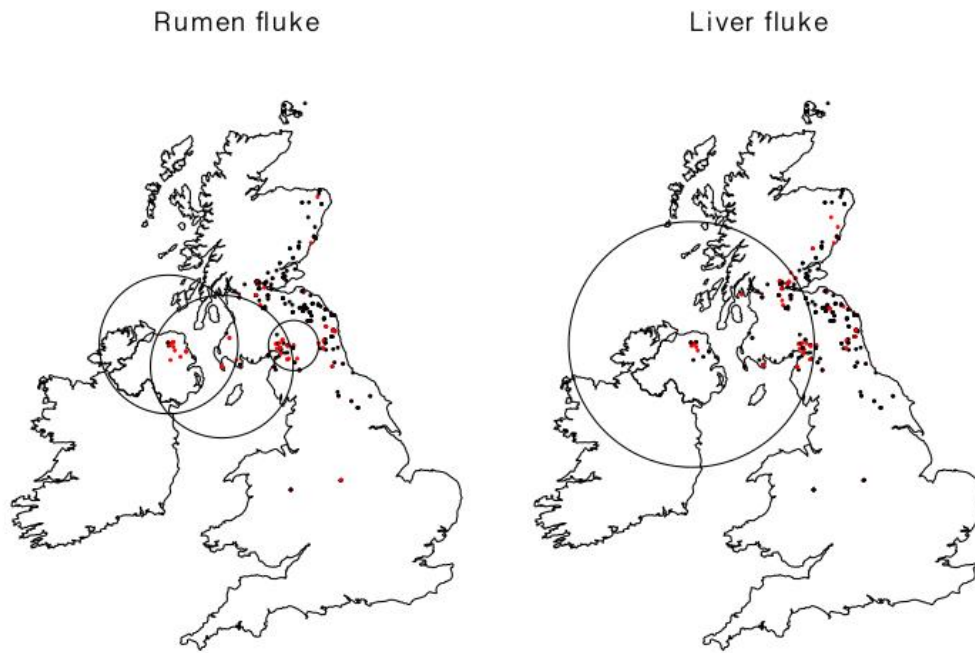


Fig. 5. Geographical distribution of rumen flukes and *F. hepatica* liver fluke-infected (red) and not infected (black) cattle. Circles indicate high risk spatial clusters identified by SatScan using the spatial scan statistic. Rumen fluke clusters (from left to right): a) relative risk 3.41, p-value <0.001; b) relative risk 3.01, p-value <0.001; c) relative risk 2.37, p-value 0.055. Liver fluke cluster: relative risk 2.41, p-value: <0.001.

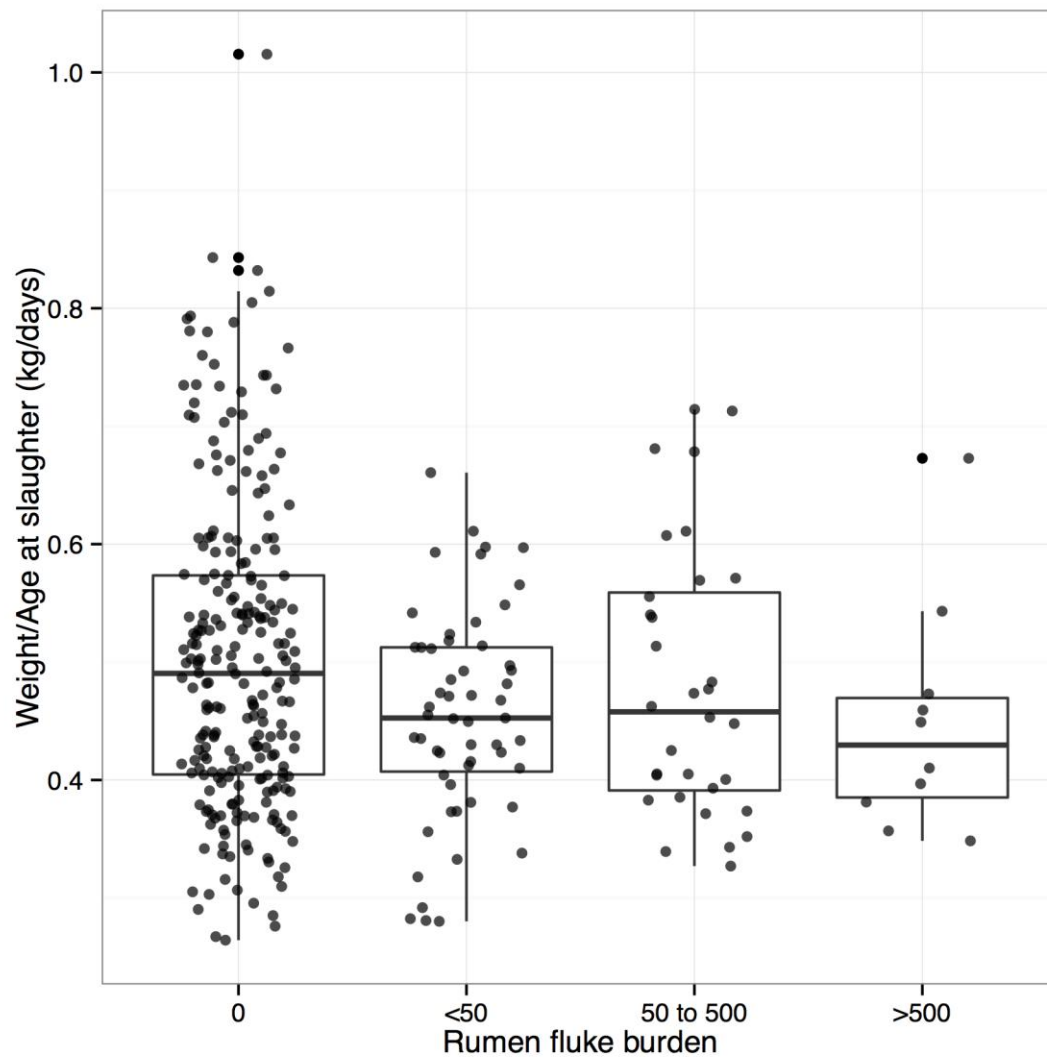


Fig. 6. Box plots of carcass weight/age of cattle Vs rumen fluke burden. Box plots to show the relationships between zero, low (<50 flukes) moderate (50 – 500 flukes) and high (>500 flukes) rumen fluke burdens and carcass weight/age of cattle at slaughter.

Table 1. Univariable regression analysis of the effects of rumen fluke and other important factors on growth rate.

Variable	Classes	All (%)	Estimate	p-value
Rumen fluke	Negative	243 (70.84)	-	-
	Positive	100 (29.15)	-0.041	0.006
Liver fluke	Negative	247 (72.01)	-	-
	Positive	96 (27.99)	-0.039	0.011
Breed	Aberdeen Angus/ X	130 (37.90)	-	-
	Charolais/ X	49 (14.29)	0.044	0.035
	Holstein Friesian	19 (5.54)	0.128	<0.001
	Limousin/ X	65 (18.95)	0.008	0.670
	Simmental X	20 (5.83)	0.002	0.933
	Other	60 (17.49)	-0.010	0.611
Period	Summer/autumn	174 (50.73)	0.012	0.380
	Winter	169 (49.27)		

Table 2. Cross tabulation of binary rumen fluke FEC results vs. identification of the parasites in the forestomach.

		Number of forestomachs		Total
		Positive	Negative	
FECs	Positive	73	7	80
	Negative	24	235	259
	Total	97	242	339

Table 3. Sensitivity (SE) and specificity (SP) estimates of rumen fluke FECs, using detection of any rumen flukes in the forestomach as a gold standard.

	SE	95% CI	SP	95% CI
Overall	0.75	0.65-0.83	0.97	0.94-0.99
Winter	0.85	0.72-0.94	0.98	0.93-0.99
Summer/autumn	0.65	0.51-0.78	0.97	0.92-0.99

Table 4. Cross tabulation of rumen fluke affected animals vs. liver fluke affected animals.

		Rumen fluke status		Total
		Positive	Negative	
Liver fluke status	Positive	44	51	95
	Negative	53	191	244
	Total	97	242	339

Table 5. Comparison of multivariable regression modeling growth rate when rumen fluke is included (top model) or not (bottom model).

Fixed effects	Random effects	AIC	Rumen fluke estimate	Rumen fluke p-value
Rumen fluke + liver fluke + breed + period	holding	-497	-0.011	0.381
Liver fluke + breed + period	holding	-505	-	-